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PRINCIPAL INVESTIGATOR: Brian I. Rini, M.D.

CONTRACTING ORGANIZATION: Cleveland Clinic Foundation
Cleveland, Ohio 44195-5001

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Brian I. Rini, M.D.

E-Mail: rinib2@ccf.org

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Background: CTLA-4 is an inhibitory molecule on T cells that induces T cell downregulation. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a growth and survival factor for dendritic cells. The safety of combining GM-CSF with CTLA-4 blockade in prostate cancer patients is being investigated in an ongoing phase I trial. Methods: Sequential cohorts of 3-6 patients receive GM-CSF 250μg/m2/d subcutaneously on days 1-14 of a 28-day cycle with escalating doses of anti-CTLA antibody on day 1 of each cycle x 4. Patients are monitored for clinical autoimmunity with T cell phenotyping performed. Results: Twenty-four patients have been treated to date. Dose-limiting toxicity (DLT) has been observed in the highest dose cohort of 3 mg/kg monthly x 4 of anti-CTLA-4 antibody in the form of autoimmune disease. Three of six patients (50%) in this cohort has a greater than 50% PSA decline, including one patient with regression of liver metastases. Expansion of monocytes / dendritic cells and upregulation of PBMC activation markers have been seen, consistent with known GM-CSF effect. A dose response relationship has been seen between anti-CTLA-4 dose and activation of both CD4+ and CD8+ T cells in the blood. These effects were increased compared to effects seen with anti-CTLA4 treatment or GM-CSF alone on a separate trial. T cell interferon-gamma production and lytic activity were also enhanced in circulating antigen-specific CD8+ T cells after this combination immunotherapy. Conclusions: CTLA-4 blockade and GM-CSF has reached a maximum tolerated dose in this patient population with autoimmunity and evidence of clinical effect.

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Introduction

This research project investigated immunotherapy for prostate cancer. Specifically, we explored the use of Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) in combination with blockade of a T cell inhibitory molecule called Cytotoxic T-Lymphocyte-Associated Molecule-4 (CTLA-4). We studied repetitive dosing of an anti-CTLA-4 antibody in combination with subcutaneous GM-CSF to determine the safety of this combination. Concomitantly, peripheral blood was being collected from patients to evaluate the immune response generated.

The original plan was to move this combination therapy into a phase II trial to look at effects on PSA and other clinical endpoints in patients failing prior vaccination for prostate cancer. This is not possible for a number of reasons. The significant autoimmune toxicity observed (which appears required for an anti-tumor effect) may preclude administration of this combination in prostate cancer patients who are often elderly with comorbidities. Further, the availability of this CTLA-4 antibody is limited. Thus, the current project will end with completion of the phase I trial noted here.

Body

Blockade of the T cell inhibitory receptor, CTLA-4, is a potent method of augmenting and potentiating T cell responses. Pre-clinical work has demonstrated enhancement of T cell responses and prostate tumor rejection via antibody-mediated blockade of CTLA-4.(1-3) Addressing a separate and similarly vital component of a coordinated immune response, systemic GM-CSF is a growth factor for dendritic cells (DC) and stimulated DC uptake of antigen to cross-prime T cells. GM-CSF has also produced PSA declines and PSA modulation in multiple prostate cancer disease states.(4, 5) A phase I trial is currently underway combining these approaches based on potential additive or synergistic activity.(6, 7) Men with hormone-refractory, metastatic prostate cancer are being treated with a constant dose and schedule of GM-CSF (250 mcg/m^2 QD d1 – d14 of a 28-day cycle) plus escalating doses (up to 3 mg/kg) of anti-CTLA-4 antibody (Ipilimumab) given on d1 of each cycle x 4.

With regard to the original proposed Statement of Work, task 1 was to determine if polyclonal T cell activation and clinical autoimmunity occur when CTLA-4 blockade is combined with GM-CSF. Twenty-four patients have been treated to date on the phase I study. The highest CTLA-4 antibody dose was 3 mg/kg monthly x 4. Clinical autoimmunity has been observed at this level in 3 of 6 patients, including grade 2 rash, grade 3 panhypopituitarism and grade 3 temporal arteritis (Table 1). No laboratory evidence of autoimmunity has been observed in any patient. Task 2 is being addressed as PSA declines are being tabulated and a manuscript will soon be written regarding the clinical results of this trial. Seven patients have demonstrated a < 50% reduction in their PSA, including 3 patients at the highest dose cohort with a greater than 50% reduction in PSA (Table 1). Task 3 from the original Statement of Work has not yet been addressed, and will not be able to be undertaken. The phase I trial has been slow to completely accrue because of safety limitations (waiting 2 months between cohorts) as required by CTEP. Further, the toxicity signal observed requires that this combination undergo further phase I testing before it could be applied more broadly in a prostate cancer population.

Table 1: Phase 1 Clinical Trial Results

Cohort level	CTLA-4 antibody dose/schedule	No. of pts.	Toxicity observed	PSA declines> 50%
1	0.5 mg/kg x 4	3	None	0/3 patients
2	1.5 mg/kg x 1, then 0.5 mg/kg x 4	6	1 CVA	0/6 patients
3	1.5 mg/kg x 4	6	Gr 3 fatigue, rash	0/6 patients
4	3 mg/kg x 1, then 1.5 mg/kg x 4	3	None	0/3 patients
5	3 mg/kg x 4	6	3 patients with autoimmune events (rash, hypopituitarism, temporal arteritis)	3/6 patients*

* including one patient with regression of liver metastases.

A dose response relationship has been seen between anti-CTLA-4 dose and activation of both $CD4^+$ and $CD8^+$ T cells in the blood. These effects were increased compared to effects seen with anti-CTLA4 treatment alone on a separate trial. T cell interferon-gamma production and lytic activity were also enhanced in circulating antigen-specific CD8+ T cells after this combination immunotherapy. This activity was more apparent at higher dose levels and appeared to persist even after discontinuation of antibody infusions.

Key Research Accomplishments

1. Anti-CTLA-4 antibody and GM-CSF have demonstrated safety when given in combination to metastatic hormone refractory prostate cancer patients.

2. Collection and immunologic assessment of baseline and serial peripheral blood samples is feasible.

3. This combination immunotherapy in metastatic hormone refractory prostate cancer patients produces expansion of activated monocytes and dendritic cells, as well as activation of an endogeneous population of cytotoxic T-lymphocytes *in vivo*.

4. PSA declines have been observed with this combination immunotherapy.

5. The highest dose level tested of this combination produces significant clinical autoimmunity which is associated with PSA declines and tumor regression

Reportable Outcomes

1. This data was a poster presentation at the ASCO 2004 annual meeting (L. Fong, B. Rini, B. Cavanaugh, E. Small. CTLA-4 Blockade-Based Immunotherapy for Prostate Cancer. Proc Am Soc Clin Oncol 22:14s, 2590, 2004).

2. This data was presented at the National Specialized Program of Research Excellence (SPORE) meeting in Baltimore, MD in July 2004. It was an oral presentation.

3. A serum repository of baseline and treatment samples for all patients is available and stored in the Immunology Core Laboratory of Dr. Larry Fong, who is performing the immunologic assays. This repository of serum will provide valuable companion data to this study, and a potential source of data for future studies.

4. This data was presented at the 2007 ASCO Prostate Cancer Symposium. (E. J. Small, V. Weinberg, B. Kavanagh, J. Valiente, B. I. Rini, L. Fong. Combination immunotherapy with GM-CSF and ipilimumab (anti-CTLA4 antibody) in patients with metastatic hormone refractory prostate cancer (oral presentation)).

Conclusions

CTLA-4 blockade-based immunotherapy in combination with GM-CSF is feasible in metastatic hormone refractory prostate cancer. The phase I study defined an MTD that was associated with PSA declines, but also significant clinical autoimmunity, precluding further phase II clinical trials at this point. Initial immunologic results suggest an effect of this therapy on both monocytes, dendritic cells, and T cells. Correlation of this response with clinical outcome is forthcoming.

Future Plans (Task 3)

The current phase I trial will be completed and clinical and immunologic data reported in manuscript form. There are no immediate plans to take this combination forward to phase II testing at this time. As noted in previous report, a trial was proposed through ECOG, but this was not supported. The data noted above in regards to toxicity are notable in light of a typical elderly prostate cancer population. As such, further development of this combination will require further phase I study in the context of a different trial.

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